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(71) Applicant *(for all designated States except US)*: THE PENN STATE RESEARCH FOUNDATION [US/US]; 304 Old Main Street, University Park, PA 16802 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: CLAWSON, Gary, A. [US/US]; 5709 Rockmere Drive, Bethesda, MD 20816 (US). PAN, Wei-Hua [CN/US]; 907 Butler Court, Hummelstown, PA 17036 (US). XIN, Ping [CN/US]; 907 Butler Court, Hummelstown, PA 17036 (US).

(74) Agent: FRASER, Janis, K.; Fish & Richardson P.C., 225 Franklin Street, Boston, MA 02110-2804 (US).

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(54) Title: RNA INTERFERENCE COMPOSITIONS AND METHODS

(57) Abstract: The invention provides isolated nucleic acids. For example, the invention provides isolated nucleic acids having at least one strand with both sense and antisense sequences that are complementary to each other. The invention also provides isolated nucleic acids having at least one strand that is a template for both sense and antisense sequences that are complementary to each other. In addition, the invention provides cells, viruses, and transgenic animals (e.g., transgenic non-human animals) containing one or more of the isolated nucleic acids provided herein as well as methods for using one or more of the isolated nucleic acids provided herein to reduce the level of an RNA (e.g., an mRNA) within a cell.

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(54) Title: RNA INTERFERENCE COMPOSITIONS AND METHODS

(57) Abstract: The invention provides isolated nucleic acids. For example, the invention provides isolated nucleic acids having at least one strand with both sense and antisense sequences that are complementary to each other. The invention also provides isolated nucleic acids having at least one strand that is a template for both sense and antisense sequences that are complementary to each other. In addition, the invention provides cells, viruses, and transgenic animals (e.g., transgenic non-human animals) containing one or more of the isolated nucleic acids provided herein as well as methods for using one or more of the isolated nucleic acids provided herein to reduce the level of an RNA (e.g., an mRNA) within a cell.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/05400

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) : C07H 21/04
US CL : 536/24.5
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. :

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Symonds et al. (US Patent Application Publication 2002/0160393) (Fig. 8C, paragraphs 91, 117, 214-217, for example)	1-6, 8, 12, 13, 15, 16, 18, 19, 56, 65
X	Yuyama et al. (1994) Nucleic Acids Res. 22:5060-5067	1, 2
X	Taira et al. (US Patent 5,500,357)	1, 2

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
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Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
Facsimile No. (571) 273-3201

Authorized officer
Louis V. Wollenberger
Telephone No. 571-272-1600

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/05400

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of any additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-33, 56-61, 65, and 66 and SEQ ID NO:1

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees

BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-33, 56-61, 65, and 66, drawn to an isolated nucleic acid comprising a strand that is a template for an RNA molecule comprising sense and antisense sequences and a cis-acting ribozyme sequence, and to compositions, cells, viruses, and transgenic animals thereof.

Group II, claim(s) 34-50, 56-61, 65, and 66, drawn to an isolated nucleic acid comprising a strand that is a template for an RNA molecule comprising sense and antisense sequences and a cis-acting ribozyme sequence, and to compositions, cells, viruses, and transgenic animals thereof.

Group III, claim(s) 51-55, 56-59, 65, and 66 drawn to an isolated RNA strand comprising sense and antisense sequences and a cis-acting ribozyme sequence.

Group IV, claim(s) 62-64, drawn to a method of identifying sequences capable of inducing RNA interference against a target mRNA.

Group V, claim(s) 67-83, drawn to an isolated nucleic acid.

Group VI, claim(s) 84-100, drawn to a nucleic acid library.

Group VII, claim(s) 101-119, drawn to an isolated nucleic acid.

Group VIII, claim(s) 120-138, drawn to a nucleic acid library.

Group IX, claim(s) 139-142, drawn to a method for making a library.

The inventions listed as Groups I-XIV do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical feature of Group I is an isolated nucleic acid that is a template for an RNA molecule comprising sense and antisense sequences and a cis-acting ribozyme, wherein said sense and antisense sequences form double-stranded RNA upon cleavage by said cis-acting ribozyme. This element is not present in any of the other groups.

The special technical feature of Group II is an isolated nucleic acid that is a template for an RNA molecule comprising sense and antisense sequences and a cis-acting ribozyme, wherein said sense and antisense sequences form single-stranded RNA upon cleavage by said cis-acting ribozyme. This element is not present in any of the other groups.

The special technical feature of Group III is an isolated RNA strand comprising sense and antisense sequences and a cis-acting ribozyme, wherein said sense and antisense sequences form single-stranded RNA upon cleavage by said cis-acting ribozyme. This element is not present in any of the other groups.

The special technical feature of Group IV is a vector preparation, wherein each vector comprises a target sequence reporter sequence fusion construct. This element is not present in any of the other groups.

The special technical feature of Groups V, VI, and VII is a dsRNA-encoding vector construct, capable of inducing RNAi, comprising two promoters linked to a nucleic acid sequence to promote transcription of both strands of said sequence, which construct is not present in any of the other groups. However, this cannot be the special technical feature under PCT Rule 13.2, because the construct is shown in the prior art. Wang et al. teach a vector comprising opposing T7 promoters flanking a nucleic acid insert so as to promote transcription of dsRNA capable of inducing RNAi (Fig. 1).

The special technical feature of Groups VIII and IX is a hairpin RNA or a template or construct encoding a hairpin RNA, which element(s) are not present in any of the other groups. However, this cannot be the special technical feature under PCT Rule 13.2, because the element(s) are shown in the prior art. Wang et al. teach a vector comprising a procyclin promoter linked to an inverted repeat sequence so as to promote transcription of a hairpin RNA capable of inducing RNAi (Fig. 1).

The nucleic acid sequences listed in Claim 7 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Claim 7 specifically claims 18 different nucleic acid sequences comprising a sequence selected from any one of SEQ ID NOS: 1...54.

This international searching authority considers that the international application does not comply with the requirements of unity of invention (Rules 13.1, 13.2 and 13.3) for the reasons indicated below.

According to the guidelines in Section (f) of Annex B of the PCT Administrative Instructions, the requirement of a technical interrelationship and the same or corresponding special technical features as defined in Rule 13.2, shall be considered to be met when the alternatives are of a similar nature.

Sections (f)(i), (ii), and (iii) of Annex B of the PCT Administrative Instructions state:

(i) When the Markush grouping is for alternatives of chemical compounds, they shall be regarded as being of a similar nature where the following criteria are fulfilled:
(A) all alternatives have a common property or activity, and
(B) (1) a common structure is present, i.e., a significant structural element is shared by all of the alternatives, or (B) (2) in cases where the common structure cannot be the unifying criteria, all alternatives belong to a recognized class of chemical compounds in the art to which the invention pertains.
(ii) In paragraph (f)(i)(B)(1), above, the words "significant structural element is shared by all of the alternatives" refer to cases where the compounds share a common chemical structure which occupies a large portion of their structures, or in case the compounds have in common only a small portion of their structures, the commonly shared structure constitutes a structurally distinctive portion in view of existing prior art, and the common structure is essential to the common property or activity. The structural element may be a single component or a combination of individual components linked together.
(iii) In paragraph (f)(i)(B)(2), above, the words "recognized class of chemical compounds" mean that there is an expectation from the knowledge in the art that members of the class will behave in the same way in the context of the claimed invention. In other words, each member could be substituted one for the other, with the expectation that the same intended result would be achieved.

In the instant case, 18 alternative polynucleotide sequence SEQ ID NOs are recited in claim 7. The alternative polynucleotides do not appear to share a common property or activity or a common structure or significant structural element or belong to a recognized class of chemical compounds, according to the criteria in B(f)(iii), above, wherein each member could be substituted for the other with the expectation that the same result would be achieved.

Each sequence appears to be structurally unique, and may therefore be expexted to have different properties in the context of the invention as a whole. For example, because each sequence is different, each sequence may be exprected to target a different and/or specific region of the same and/or different target gene(s), and, absent evidence to the contrary, each sequence may be expected to modulate (either increase or decrease) the expression of the target gene to varying degrees. Thus, each member of the class cannot be substituted, one for the other, with the expectation that each will perform the same function with the same efficiency.

Further, although the instant sequences target the same gene, the sequences do not meet the criteria of (B)(1), as they do not appear to share, one with another, a common core structure. Accordingly, unity of invention between the sequences of the instant application is lacking and each sequence claimed is considered to constitute a special technical feature.

Applicants will obtain a search of the first sequence listed claim 7 in the instant application.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/05400

Continuation of B. FIELDS SEARCHED Item 3:
STN(Medline, Biosis, CaPlus, Embase, Registry): PubMed; STIC-Biotech Sequence search of commercial databases for oligonucleotides comprising SEQ ID NO:1